

# Detection and Prediction of Acute Heart Transplant Rejection With the Myocardial $T_2$ Determination Provided by a Black-Blood Magnetic Resonance Imaging Sequence

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<b>OBJECTIVES</b>	This study aimed to determine whether the myocardial $T_2$ relaxation time, determined using a black-blood magnetic resonance imaging (MRI) sequence, could predict acute heart transplant rejection.
<b>BACKGROUND</b>	The use of black-blood MRI sequences allows suppression of the confusing influence of blood signal when myocardial $T_2$ is calculated to detect myocardial edema.
<b>METHODS</b>	A total of 123 investigations, including cardiac MRI and myocardial biopsy, were performed $8 \pm 11$ months after heart transplantation. Myocardial $T_2$ was determined using an original inversion-recovery/spin-echo sequence.
<b>RESULTS</b>	A higher than normal $T_2$ ( $\geq 56$ ms) allowed an accurate detection of the moderate acute rejections evidenced at baseline biopsy ( $\geq$ International Society for Heart and Lung Transplantation grade 2): sensitivity, 89% and specificity, 70% ( $p < 0.0001$ ). $T_2$ was increased in grade 2 ( $n = 11$ ) compared with grade 0 ( $n = 49$ , $p < 0.05$ ), grade 1A ( $n = 34$ , $p < 0.05$ ) and grade 1B ( $n = 21$ , $p < 0.05$ ); $T_2$ was further increased in grade 3 ( $n = 8$ ) compared with grade 2 ( $p < 0.05$ ). In addition, in patients without rejection equal to or greater than grade 2 at baseline, a $T_2$ higher than normal ( $\geq 56$ ms) was correlated with the subsequent occurrence of equal or greater than grade 2 rejection within the next three months: sensitivity 63% (12/19) and specificity 78% (64/82) ( $p = 0.001$ ).
<b>CONCLUSIONS</b>	Myocardial $T_2$ , determined using a black-blood MRI sequence, is sufficiently sensitive to identify most of the moderate acute rejections documented with biopsy at the same time, but is also a predictor of the subsequent occurrence of such biopsy-defined rejections. (J Am Coll Cardiol 2001;37:825–31) © 2001 by the American College of Cardiology

Survival after cardiac transplantation has improved thanks to the use of more specific immunosuppressive drugs and the extensive use of endomyocardial biopsies, which allow acute rejection to be detected before allograft dysfunction (1). However, endomyocardial biopsy leads to nonnegligible patient risk and discomfort (2) and has some limitations, such as the possibility of false negative results (3) and variability of interpretation (4). Therefore, looking for alternative noninvasive techniques is clinically warranted.

The measurement of the magnetic resonance [ $H^+$ ] relaxation times allows a noninvasive detection of myocardial edema and has been shown to detect acute allograft rejection in animals (5–10). Preliminary reports using conventional magnetic resonance imaging (MRI) sequences in humans have, however, given discrepant results (11–14). We previously reported that [ $H^+$ ] myocardial relaxation times in

healthy subjects could be precisely determined by combined spin-echo and inversion-recovery sequences on a moderate field strength (0.5 T) magnet (15,16). Moreover, in a preliminary report, we observed that a single spin-echo/inversion-recovery (“black-blood”) sequence, allowing a rapid and precise determination of the myocardial transversal relaxation time ( $T_2$ ), was able to detect episodes of acute heart transplant rejection in humans (17). The aim of this current study was to determine the usefulness of this MRI procedure in the detection and prediction of biopsy-defined acute heart transplant rejection.

## METHODS

**Study population.** At our institution during a four-year period, we had the opportunity to perform two MRI examinations weekly in patients with heart transplant. The patients were referred to MRI on the basis that: 1) they were hospitalized either for a suspected rejection or for scheduled systematic investigations; 2) they had no evidence of either myocarditis or myocardial infarction; 3) they had no con-

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# Abbreviations and Acronyms

IVRT	= isovolumetric relaxation time
LV	= left ventricle
MRI	= magnetic resonance imaging
PHT	= pressure half-time
SD	= standard deviation
T <sub>2</sub>	= [H <sup>+</sup> ] transversal relaxation time

traindication for MRI; and 4) they gave informed consent to undergo this additional investigation. When more than two patients could be selected, only the two believed to have the higher probability of acute rejection were referred to MRI: those having signs suggestive of rejection at clinical examinations (fever, fatigue) or at Doppler-echocardiography (left ventricle [LV] systolic dysfunction, abnormal diastolic pattern) or, in the absence of any sign of rejection, those who had the shorter delay time from transplantation.

For the present study, which was undertaken to analyze the relationship between the results from MRI and those from myocardial biopsy, MRI investigations were retrospectively selected according to the following criteria: 1) a myocardial biopsy was obtained within one week of MRI; 2) no intravenous treatment for acute rejection had been given in the week preceding MRI or in the period between MRI and myocardial biopsy; and 3) the patients were not identified as having a chronic transplant rejection at the time of these investigations.

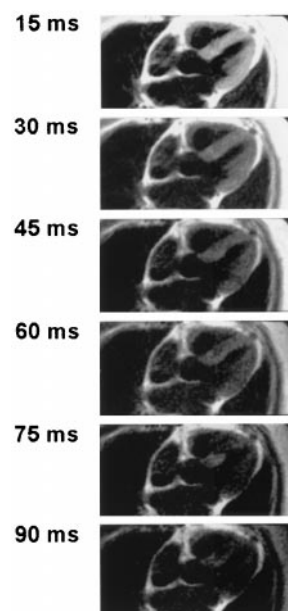
According to these criteria, 131 MRI procedures were selected, among which eight were excluded because of poor image quality (6%). Finally, 123 MRI procedures performed in 68 patients were considered for this study. Thirty-five patients had a single procedure and 33 had from two to four procedures.

Twenty MRIs were performed in women (16%) and mean age of the patients was  $50 \pm 13$  years (from 16 to 69 years) at the time of MRI. The mean time delay between MRI and myocardial biopsy was  $1 \pm 3$  days. MRI was performed in patients with suspected acute rejection (on the basis of clinical or echographic data) in 49 cases (40%) and as a systematic control in 74 (60%). Mean delay between heart transplantation and MRI was  $8 \pm 11$  months (from one week to six years) and MRI was performed within the year following transplantation in 101 cases (82%).

**Magnetic resonance imaging.** Magnetic resonance imaging was carried out on a whole-body magnet operating at a field strength of 0.5 T (MRmax, General Electric, Milwaukee, Wisconsin). As previously described (15–17), the imaging protocol started with multislice pilot scans oriented in the coronal plane, and thereafter along the long axis of the left ventricle.

A single slice, which allowed a clear view of the intraventricular septum and which was the most central among the left ventricular oblique views, was chosen for the T<sub>2</sub> determination sequence.

The black-blood sequence was constituted by a 180°



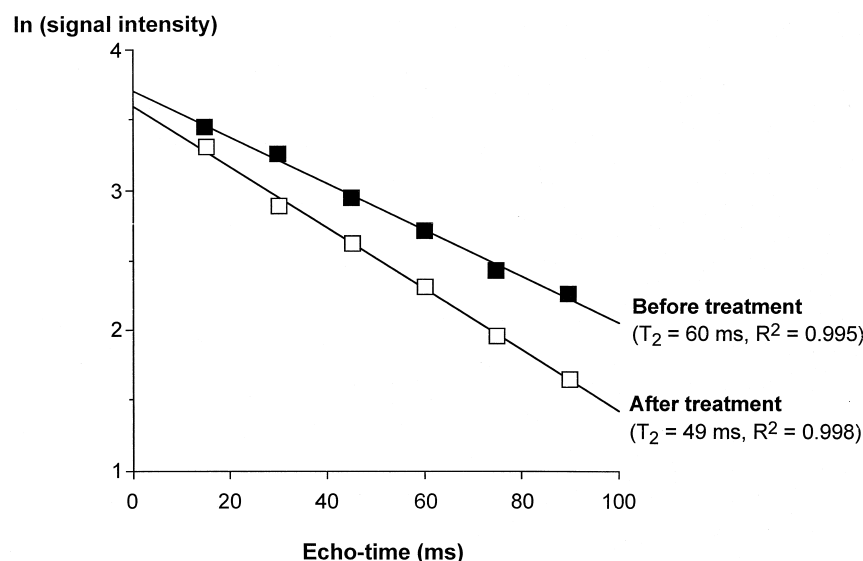
**Figure 1.** Example of images obtained using the black blood sequence with echo images at 15, 30, 45, 60, 75 and 90 ms. The ratios, calculated for each echo image between the myocardial signal from septal wall and the blood signal from left ventricle cavity, were 9.7 at 15 ms, 5.8 at 30 ms, 3.9 at 45 ms, 3.4 at 60 ms, 2.6 at 75 ms and 2.1 at 90 ms.

inversion nonselective pulse followed by a conventional spin-echo selective pulse sequence. An inversion time of 800 ms was chosen to withdraw most of the blood-related signal. This time value was determined by the equation:  $t = \ln(2) \times T_1$ , where  $T_1$  represents the longitudinal relaxation time of blood at 37° C and at a field strength of 0.5 T (18). Six echoes were acquired with echo times (TE) of 15, 30, 45, 60, 75 and 90 ms. Imaging was performed with a field of view of 42 cm, a scanning matrix of  $224 \times 64$  and a “no phase wrap” function. In order to increase the amount of myocardial signal (which was decreased by the use of the inversion pulse), a slice thickness of 20 mm was used, image acquisition was gated to the systolic period at the falling edge of the T wave (to image a broader myocardial thickness) and as many as four excitations were recorded. The repetition time was forced to fall in the range 2,000 to 3,000 ms. An example of the images provided by the black-blood sequence is given in Figure 1.

Two to three circular regions of interest, including the external borders of the myocardium, were placed along the intraventricular septum on the first echo image and reproduced unchanged on other echo images. To avoid the confusing influence of signal from surrounding fat, the other left ventricular walls (apical and lateral) were not analyzed.

The T<sub>2</sub> calculation was performed with a standard linearized least-square fit applied on the myocardial signal measured on the echo images and based upon the equation  $M(TE) = M_0 e^{-TE/T_2}$ , TE being the echo time and M(TE) the averaged signal from all regions of interest of the corresponding TE image. Examples of linear fits are given in Figure 2.

For the 47 initial patients, an additional determination of



**Figure 2.** Examples of linear fits allowing the myocardial  $T_2$  determination and obtained in the same patient (1) (black squares) at the time of a grade 3 rejection at biopsy and (2) (white squares) one week later, after the intravenous treatment of acute rejection. The fit is applied on the linear decrease with time of the logarithm of myocardial signal intensity.  $T_2$  is calculated as the inverse of the slope.  $T_2 = [H^+]$  transversal relaxation time.

$T_2$  was obtained with a standard spin-echo sequence applied on the same slice, and thus without inversion pulse and with more conventional parameters: slice thickness of 10 mm, two excitations, scanning matrix of  $224 \times 128$  and four echoes acquired with echo times of 15, 30, 45 and 60 ms.

Finally, the reproducibility of  $T_2$  measurement has been assessed in 23 cases where two consecutive black-blood sequences have been performed.

**Doppler studies.** The results from transmitral Doppler could be analyzed in 105 cases (85%) where a Doppler-echocardiographic study was performed within 48 h of MRI without prior intravenous treatment for acute rejection and where at least one previous Doppler analysis could be used as a normal reference.

Using a 3 MHz combined imaging and Doppler transducer (Vingmed CFM 750, Horten, Norway), pulsed-wave transmitral Doppler tracings were recorded from the apical four-chamber view by experienced physicians. The sample volume was positioned at the level of the tips of mitral leaflets and two diastolic parameters were determined (19): 1) the isovolumetric relaxation time (IVRT), calculated as the time interval between aortic closure and the onset of mitral flow, and 2) the pressure half-time of early mitral flow deceleration (PHT).

An abnormal diastolic pattern, suggestive of acute rejection, was defined as a  $>15\%$  decrease in either PHT or IVRT when compared with normal reference values (19). For each individual, the normal reference value was determined by averaging the last values (up to three) that had been calculated in the absence of suspected rejection.

**Transvenous endomyocardial biopsies.** Transvenous endomyocardial biopsies were performed only as clinically indicated for systematic control or in case of suspected rejection. The systematic controls were performed at the

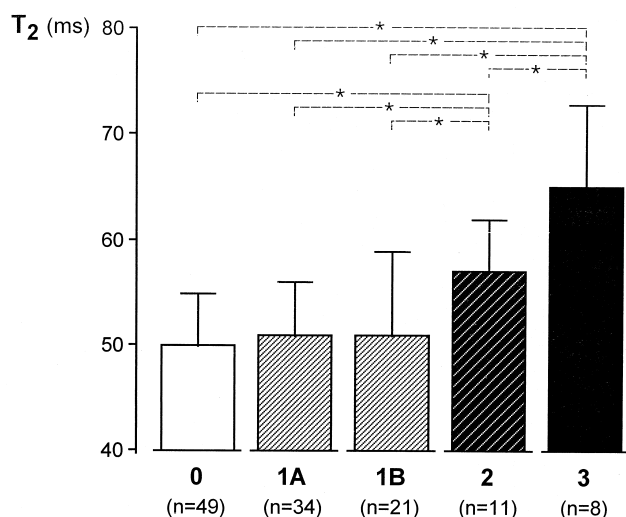
following rates: at two-week intervals for the first four months following transplantation, then at monthly intervals until the end of the first year, and finally at two-month intervals during the second year. Using an extensively described method (1), at least four specimens of right ventricular tissue were obtained. Biopsies were interpreted by experienced pathologists and graded according to ISHLT (20): no evidence of rejection (grade 0); focal (grade 1A) or diffuse (grade 1B) mild signs of rejection; focal (grade 2), multifocal (grade 3A) or diffuse (grade 3B) moderate signs of rejection; and severe signs of rejection (grade 4).

**Statistical analysis.** Comparisons between discrete variables and comparisons between proportions were made using the Fisher exact test. Continuous variables were expressed as mean  $\pm$  standard deviation and compared with nonparametric tests: Mann-Whitney  $U$  and Wilcoxon tests were used for unpaired and paired two-group comparisons, respectively, and multiple unpaired comparisons were performed using the Kruskal Wallis analysis of variance. For all tests, a  $p$  value  $< 0.05$  was considered to be indicative of a significant difference.

## RESULTS

**Detection of acute graft rejection.** On the biopsies performed at baseline, there were 49 ISHLT grade 0 (40%), 34 grade 1A (28%), 21 grade 1B (17%) and 19 (15%)  $\geq$  grade 2 rejections: 11 grade 2, seven grade 3A and one grade 3B.

As illustrated in Figure 3, the averaged  $T_2$  values were similar between grade 0 ( $50 \pm 5$  ms), grade 1A ( $51 \pm 5$  ms) and grade 1B ( $51 \pm 8$  ms). In contrast,  $T_2$  was significantly higher in grade 2 ( $57 \pm 5$  ms) than in either grade 0, grade



**Figure 3.** Comparison of the averaged values of myocardial  $T_2$  ( $\pm$  SD), calculated in groups stratified according to the results of myocardial biopsies performed at baseline (ISHLT grade 0, 1A, 1B, 2 and 3).  $p < 0.05$ , for the adjusted comparisons among the five groups.  $T_2 = [H^+]$  transversal relaxation time.

1A or grade 1B (all  $p < 0.05$ ); and  $T_2$  was even higher in grade 3 ( $65 \pm 8$  ms) than in grade 2 rejection ( $p < 0.05$ ).

$T_2$  was markedly higher in patients with than in those without moderate ( $\geq$ grade 2) rejection at biopsy ( $60 \pm 7$  ms vs.  $51 \pm 6$  ms,  $p = 0.0001$ ) and a  $T_2 \geq 2$  SD of normal ( $\geq 56$  ms [15]) was the best cutoff value to separate patients with and without  $\geq$ grade 2 rejection: sensitivity was 89% (17/19) and specificity 70% (73/104). This criterion was associated with a high negative predictive value (97%) but with a much lower positive predictive value (35%) in the detection of  $\geq$ grade 2 rejection.

As detailed in Table 1, no other analyzed variable was significantly related to the presence of  $\geq$ grade 2 rejection at biopsy, except for the IVRT, determined with transmitral Doppler, which was lower and showed a higher decrease in  $\geq$ grade 2 rejection compared with previous analyses.

Among the 47 initial patients for whom  $T_2$  had additionally been determined using a conventional spin-echo sequence, eight had a  $\geq$ grade 2 rejection. Compared with the  $T_2$  values provided by the black blood sequence, those provided by the conventional sequence were higher and showed a higher inter-individual variability which could be documented both in the 8 patients who had a  $\geq$ grade 2 rejection ( $66 \pm 13$  ms vs.  $61 \pm 6$  ms,  $p = 0.04$ ) and in the 39 others ( $53 \pm 12$  ms vs.  $49 \pm 6$  ms,  $p = 0.01$ ). When  $T_2$  was compared between patients with and those without  $\geq$ grade 2 rejection, the difference was more pronounced with the black-blood sequence ( $61 \pm 6$  ms vs.  $49 \pm 6$  ms,  $p < 0.0001$ ) than with the conventional sequence ( $66 \pm 13$  ms vs.  $53 \pm 12$  ms,  $p = 0.01$ ), though the relationship was statistically significant for both sequences.

Finally, in the 23 cases where two consecutive black-blood sequences had been performed, the averaged differ-

**Table 1.** Comparison of Data From Clinical History, MRI and Transmitral Doppler, Between Patients With and Those Without  $\geq$ Grade 2 Rejection on the Myocardial Biopsy Performed at Baseline

	Myocardial Biopsy at Baseline		p Value
	$\geq$ Grade 2 (n = 19)	< Grade 2 (n = 104)	
Clinical data			
Age (yrs)	46 $\pm$ 17	51 $\pm$ 12	NS
Female gender	3 (16%)	17 (16%)	NS
Delay-time from transplantation (months)	12 $\pm$ 19	10 $\pm$ 18	NS
Recent history of acute rejection ( $\leq 1$ month)	3 (16%)	10 (10%)	NS
Clinical signs of acute rejection	8 (42%)	30 (29%)	NS
Daily dose of corticosteroids (mg of prednisone-equivalent)	27 $\pm$ 30	26 $\pm$ 25	NS
Myocardial $T_2$ at MRI			
Value (ms)	60 $\pm$ 7	51 $\pm$ 6	0.0001
$\geq 2$ SD of normal ( $\geq 56$ ms)	17 (89%)	31 (30%)	<0.0001
$\geq 1$ SD of normal ( $\geq 53$ ms)	18 (95%)	52 (50%)	0.0002
Transmitral Doppler	(n = 18)	(n = 87)	
Pressure half time			
Value (ms)	46 $\pm$ 21	41 $\pm$ 16	NS
Change (%)	3 $\pm$ 28	-5 $\pm$ 19	NS
Isovolumetric relaxation time			
Value (ms)	86 $\pm$ 12	93 $\pm$ 13	0.03
Change (%)	-9 $\pm$ 12	-2 $\pm$ 12	0.01

NS = nonsignificant; SD = standard deviation;  $T_2 = [H^+]$  transversal relaxation time.

ence in  $T_2$  value between the two measurements was found to be  $1.5 \pm 1.7$  ms.

**Follow-up analysis.** In order to determine whether the number of false positive results in the detection of moderate rejections ( $T_2 \geq 56$  ms but no  $\geq$ grade 2 rejection) might correspond to an early stage of such biopsy-defined rejections, the subsequent occurrence of  $\geq$ grade 2 rejection over the following three months was sought in the patients who had no  $\geq$ grade 2 rejection at baseline.

During this period, three patients died without documented acute rejection and were excluded from the analysis: two with normal  $T_2$  ( $< 56$  ms) died of a pulmonary cancer and of a pulmonary infection, respectively, and one with an abnormal  $T_2$  ( $\geq 56$  ms) had an unexplained sudden death.

Among the 101 cases that were finally considered for the follow-up analysis, 19 had a  $\geq$ grade 2 rejection that was documented during the three-month period by an additional biopsy (12 grade 2 and seven grade 3 rejections).

This additional biopsy was performed 15 to 92 days after MRI (mean  $50 \pm 27$  days) as systematic control in 10 cases (53%) and because of suspected acute rejection (on the basis of clinical or Doppler data) in nine cases (47%).

In none of the remaining 82 cases was a  $\geq$ grade 2 rejection documented at biopsy during the three months of



**Table 2.** Comparison of the Baseline Data, in the Patients Without  $\geq$ Grade 2 Rejection at Baseline, Between Those Who Had and Those Who Had No  $\geq$ Grade 2 Rejection Over the Subsequent Three-Month Follow-up Period

	Follow-up		p Value
	Rejection (n = 19)	No Rejection (n = 82)	
Clinical data			
Age (yrs)	53 $\pm$ 11	50 $\pm$ 12	NS
Female gender	1 (5%)	16 (20%)	NS
Delay time from transplantation (months)	3 $\pm$ 3	12 $\pm$ 20	< 0.05
Recent history of acute rejection ( $\leq$ 1 month)	0 (0%)	10 (12%)	NS
Clinical signs of acute rejection	4 (21%)	25 (30%)	NS
Daily dose of corticosteroids (mg of prednisone-equivalent)	30 $\pm$ 17	26 $\pm$ 27	NS
Myocardial biopsy			
Grade 0	8 (42%)	39 (48%)	NS
Grade 1A	7 (37%)	26 (32%)	NS
Grade 1B	4 (21%)	17 (21%)	NS
Myocardial T <sub>2</sub> at MRI			
Value (ms)	54 $\pm$ 4	50 $\pm$ 6	< 0.05
$\geq$ 2 SD of normal ( $\geq$ 56 ms)	12 (63%)	18 (22%)	< 0.05
$\geq$ 1 SD of normal ( $\geq$ 53 ms)	16 (84%)	34 (41%)	< 0.05
Transmitral Doppler	(n = 15)	(n = 69)	
Pressure half time			
Value (ms)	39 $\pm$ 17	41 $\pm$ 16	NS
Change (%)	-7 $\pm$ 15	-5 $\pm$ 20	NS
Isovolumetric relaxation time			
Value (ms)	98 $\pm$ 12	92 $\pm$ 13	NS
Change (%)	2 $\pm$ 12	-3 $\pm$ 12	NS

NS = nonsignificant; SD = standard deviation; T<sub>2</sub> = [H<sup>+</sup>] transversal relaxation time.

follow-up. In 68 cases (83%), this absence of rejection could be ascertained by a myocardial biopsy performed after the end of the follow-up period (on average, 20  $\pm$  20 days later). In the remaining 14 cases, no additional biopsy was performed after the follow-up period (because this was not considered to be clinically warranted), but the absence of any  $\geq$ grade 2 rejection was strongly suggested by an event-free outcome of at least six months from MRI.

Myocardial T<sub>2</sub> was significantly higher in patients with than in those without  $\geq$ grade 2 rejection during the three-month follow-up period (54  $\pm$  4 ms vs. 50  $\pm$  6 ms,  $p$  < 0.05). As detailed in Table 2, neither clinical nor Doppler parameters were significant predictors, except the time delay between MRI and transplantation, which was shorter in patients with than in those without  $\geq$ grade 2 rejection at follow-up (3  $\pm$  3 months vs. 12  $\pm$  20 months,  $p$  < 0.05).

A T<sub>2</sub>  $\geq$ 2 SD of normal ( $\geq$ 56 ms [15]) was correlated ( $p$  < 0.05) with the occurrence of a  $\geq$ grade 2 rejection within the three following months: the specificity was 78% (64/82) and the sensitivity 63% (12/19). However, the sensitivity was clearly time-dependent because 80% (4/5) of the acute rejection detected in the first month of follow-up had an abnormal T<sub>2</sub> at baseline, whereas this was the case for 67% (4/6) and 50% (4/8) of the rejections detected during the second and the third months, respectively.

Finally, the criterion of an abnormal T<sub>2</sub> ( $\geq$ 56 ms) was strongly associated with the occurrence of a  $\geq$ grade 2 rejection either at baseline or in the following three months,

this criterion having a high negative predictive value (88%) and a lower positive predictive value (62%).

**Significance of an abnormal T<sub>2</sub> value in the absence of any  $\geq$ grade 2 rejection at baseline and follow-up.** Baseline data from patients who had no  $\geq$ grade 2 rejection both at baseline and at follow-up were compared between those with and without an abnormal T<sub>2</sub> value at baseline (Table 3). The only significant variable was the IVRT, which was lower and showed a greater change compared with previous analyses when T<sub>2</sub> was abnormal. When T<sub>2</sub> was abnormal, the IVRT was significantly decreased compared with normal individual reference values (-9  $\pm$  15%,  $p$  < 0.05); when T<sub>2</sub> was normal, by contrast, the IVRT was not significantly changed (-1  $\pm$  10%, NS).

## DISCUSSION

The noninvasive detection of acute heart transplant rejection remains a clinical challenge. The present study reports the results provided in this setting by a rapid (<30 min) MRI procedure allowing a precise determination of the myocardial T<sub>2</sub>.

**Detection of acute rejection.** Although the T<sub>2</sub> values were not significantly modified by the presence of mild signs of rejection at biopsy (grade 1), there was an increase of T<sub>2</sub> starting at the level of focal moderate signs of rejection (grade 2); this increase was even higher in cases of grade 3 rejection.

The detection of moderate forms (grades 2 and 3) is

**Table 3.** Comparison of the Baseline Data, in the Patients Without  $\geq$ Grade 2 Rejection Both at Baseline and at Follow-up, Between Those Who Had and Those Who Had No Abnormal  $T_2$  Value ( $\geq 56$  ms)

	Myocardial $T_2$		p Value
	Abnormal (n = 18)	Normal (n = 64)	
Clinical data			
Age (yrs)	49 $\pm$ 12	51 $\pm$ 12	NS
Female gender	5 (28%)	11 (17%)	NS
Delay-time from transplantation (months)	14 $\pm$ 18	11 $\pm$ 20	NS
Recent history of acute rejection ( $\leq 1$ month)	1 (6%)	9 (14%)	NS
Clinical signs of acute rejection	3 (17%)	22 (34%)	NS
Daily dose of corticosteroids (mg of prednisone-equivalent)	26 $\pm$ 30	26 $\pm$ 27	NS
Myocardial biopsy			
Grade 0	5 (28%)	34 (53%)	NS
Grade 1A	9 (50%)	17 (27%)	NS
Grade 1B	4 (22%)	13 (20%)	NS
Transmitral Doppler Pressure half time	(n = 17)	(n = 52)	
Value (ms)	41 $\pm$ 20	41 $\pm$ 14	NS
Change (%)	-12 $\pm$ 28	-4 $\pm$ 17	NS
Isovolumetric relaxation time			
Value (ms)	86 $\pm$ 14	95 $\pm$ 12	< 0.05
Change (%)	-9 $\pm$ 14	-1 $\pm$ 10	< 0.05

NS = nonsignificant; SD = standard deviation;  $T_2$  =  $[H^+]$  transversal relaxation time.

clinically relevant because an intravenous treatment is recommended in this setting (21). The criterion of a clearly abnormal  $T_2$  ( $\geq 2$  SD) was sufficiently sensitive to detect most cases with moderate rejection at biopsy (89%). In clinical practice, this high sensitivity might be useful in selecting for endomyocardial biopsy only those patients with an abnormal  $T_2$  value.

In addition, we have observed that compared with the  $T_2$  values provided by a conventional spin-echo sequence, those provided by the black-blood sequence were shorter, exhibited fewer interindividual variations and were more strongly correlated to the presence of a  $\geq$ grade 2 rejection. The superiority of the black-blood sequence can be explained by the suppression of the blood signal and its confounding effect on myocardial  $T_2$  measurement. Under conventional spin-echo sequence, by contrast, the myocardial signal may include variable proportions of signal related to blood, such as that arising from myocardial capillaries or from ventricular cavities (diffusing signal or flow artifacts), and it is likely that this inclusion may lead to various increases in the calculated values of myocardial  $T_2$ .

**Prediction of acute rejection.** In a second analysis, we tried to determine whether the number of cases with an abnormal  $T_2$ , but without  $\geq$ grade 2 rejection at baseline biopsy, might correspond to an early stage of  $\geq$ grade 2 rejection. In this analysis, a  $T_2$  higher than normal was highly correlated with the subsequent occurrence of  $\geq$ grade

2 rejection, and though the sensitivity was only 63% for the entire follow-up period, it must be considered that this percentage was markedly time-dependent, reaching as much as 80% for the first month following MRI.

By contrast, the presence of mild signs of rejection at baseline biopsy, as well as the results from transmitral Doppler, were unrelated to the subsequent occurrence of  $\geq$ grade 2 rejection. The only other baseline parameter that was a significant predictor was the time delay from transplantation, a point easily explained because the higher rates of acute rejection are known to occur within the first months following transplantation (22). Nevertheless, this study shows that MRI has the capability to predict the subsequent occurrence of moderate acute rejection as defined by biopsy, an exciting property that might be useful in patient management.

**Significance of an abnormal  $T_2$  in the absence of any  $\geq$ grade 2 rejection.** In as many as 38% of cases with abnormal  $T_2$ , a moderate-to-severe rejection was not documented by biopsy at baseline or follow-up. To our mind, however, this point does not constitute a strong limitation to the use of MRI. This is first because myocardial biopsy is far from a perfect standard in this setting (given its variability of interpretation [4] and nonnegligible rate of false negative results [3]), and therefore acute rejection may not be excluded in all cases with abnormal  $T_2$  but no evidence of rejection at biopsy. Second, our Doppler data strongly support the hypothesis of the presence of a real myocardial edema in patients having an abnormal  $T_2$  but no biopsy-defined rejection. Indeed, these patients also had evidence of a LV diastolic dysfunction on the basis of IVRT analysis. The mechanism of this possible edema, as well as its long-term clinical consequences, remains to be determined.

**Limitations of the study.** Because of an insufficient availability of the MRI apparatus, the myocardial  $T_2$  could not be determined in all transplanted patients having a myocardial biopsy. Therefore, MRI was performed preferentially in patients who had a suspected rejection on the basis of results from clinical and Doppler echographic examinations. This constitutes a situation where the use of an alternative noninvasive technique, able to replace myocardial biopsy, should be particularly helpful. However, it is unlikely that this way to select patients might have had any important impact on our results.

In addition, transmitral Doppler was not systematically performed as part of the clinical study. Therefore, contrary to MRI that were analyzed by a single observer, Doppler data were collected by several physicians during routine examinations. It is possible that this point might explain the relatively disappointing results provided by transmitral Doppler and especially by the PHT determination. However, our Doppler results are in agreement with those from several studies where IVRT, but not PHT, has been shown to correlate with biopsy-defined rejection (19,23).

Another point was that we have used a 0.5 T imager,

whereas an enhanced image quality may be provided by higher field imagers. Further studies will be required to determine whether the use of high-field imagers may enhance the results provided by black-blood MRI sequences in this setting.

Finally, because this study included only patients having a myocardial biopsy, and because biopsies are generally performed early after transplantation, our patients were investigated at short term from transplantation (eight months on average). Therefore, the results of MRI remain to be evaluated in older heart transplants.

**Conclusion.** In this population of patients with heart transplant, the presence of an abnormal  $T_2$  value, as that determined using a black-blood MRI sequence, could identify most of the moderate acute rejections documented with biopsy performed at the same time, suggesting that routine myocardial biopsy might be avoided in patients with normal  $T_2$  value. An abnormal  $T_2$  value was also a strong predictor of the subsequent occurrence of biopsy-defined acute rejections in the following months, original information that might be helpful in patient management.

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